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EVALUATION OF A NEW MOLLUSCICIDE FOR COUNTERACTING THE INTERMEDIATE SNAIL HOST OF *SCHISTOSOMA JAPONICUM*

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ABSTRACT

A novel molluscicide, derived from niclosamide, the salt of quinoid-2',5-dichloro-4'-nitrosalicylanilide (LDS) was recently developed in China. The molluscicidal activity of LDS was compared with the commonly used molluscicide niclosamide (WPN) by immersion at seven concentrations; 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mg/L, and with spraying and powdering methods (dosages of 0.2, 0.4, and 0.8 g/m^2) in the laboratory and the field. We recorded mortality at 1 d, 3 d, and 7 d. The results showed that at 0.4 mg/L immersion exposure for 72 hours, snail mortalities for LDS and WPN were 100% and 96.70% respectively in the lab, and 100% and 95.33 ± 1.15% respectively in the field. With a dosage of 0.8 g/m^2 exposure for 7 d by spraying, snail mortalities for LDS and WPN were 100% and 97.33 ± 2.30% respectively in the lab, and 99.27 ± 1.27% and 97.47 ± 3.11% in the field. With a dosage of 0.8 g/m^2 exposure at 7 d by powdering, snail mortalities for LDS and WPN were both 98% in the lab, and 100% in the field. These results from three different methods show that molluscicidal effects were similar in the lab and the field. However, LDS is much cheaper than WPN, and LDS is less toxic to fish than WPN. Therefore, LDS might be more useful than WPN for controlling snails in endemic areas of schistosomiasis in China.

Key words: China, *Oncomelania*, schistosomiasis, molluscicide, niclosamide, WPN, LDS, snail control.

INTRODUCTION

Schistosoma japonicum is a serious epidemic disease in East and Southeast Asia, including China, Indonesia, Japan and Philippines. *Oncomelania hupensis* is the unique snail intermediate host of *Schistosoma japonicum*. Currently, the major commercial chemical molluscicide niclosamide (WPN) is applied in schistosomiasis foci for snail control worldwide (MOH, 2000; Takougang et al., 2007; Lv et al., 2006; Coura-Filho et al., 1992; Meyer-Lassen et al., 1994; Graebing et al., 2004a). Since WPN was recommended by the World Health Organization (WHO, 1985, 1993, 2002), a 50% wettable powder WPN is commonly used to kill *Oncomelania hupensis* snails in China (Mao, 1990; Zhou et al., 2005; NSMC, 2006). However, due to WPN's shortcomings of considerable cost to control snails and also toxicity to fish, and the fact that it precipitates rapidly, application of WPN has been restricted in some economically poor areas and aquaculture sites in China. Recently, there has been considerable exploratory research on molluscicides involving both chemicals and plants (Zhu, 1984; Liu et al., 2000; Wei et al., 2001; Xu et al., 2003, 2004; Yuan et al., 2005a). A new molluscicide named LDS, the salt of quinoid-2',5-dichloro-4'-nitrosalicylanilide, derived from niclosamide, has been developed. The purpose of this paper is to compare the molluscicidal effects of LDS and WPN.

MATERIAL AND METHODS

Experimental Materials

Molluscicides: A 10% wettable powder of LDS was obtained from the Tongji Medical School, Huazhong University of Science and Technology. The 50% wettable powder of niclosamide ethanolamine salt (WPN) was purchased from

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TABLE 1. Percent mortality for time and concentration of two drugs investigated by immersion tests in the lab.

	LDS			WPN		
Active Concentration (mg/L)	24 h	48 h	72 h	24 h	48 h	72 h
0.05	5.57 ± 5.10	25.57 ± 1.96	33.33 ± 3.35	10.00 ± 3.30	35.93 ± 2.37	48.90 ± 15.42
0.1	15.57 ± 1.96	58.90 ± 6.97	94.43 ± 1.96	15.57 ± 1.96	44.43 ± 1.96	87.77 ± 5.08
0.2	27.80 ± 1.91	87.77 ± 12.64	98.90 ± 1.91	22.20 ± 1.91	63.33 ± 3.35	92.23 ± 3.87
0.4	64.77 ± 2.54	100.00 ± 0.00	100.00 ± 0.00	35.97 ± 2.39	70.20 ± 0.35	96.70 ± 0.00
0.8	86.37 ± 0.29	100.00 ± 0.00	100.00 ± 0.00	42.20 ± 1.91	95.57 ± 1.96	100.00 ± 0.00
1.6	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	46.70 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
3.2	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	56.70 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Control	2.87 ± 0.51	3.53 ± 0.68	5.70 ± 1.00	2.87 ± 0.51	3.53 ± 0.68	5.70 ± 0.00

the Sichuan Academy of Chemical Industry and Design.

Test Containers: Unglazed earthen bowls (20 cm diameter and 7 cm height) were used. Soil containing rich humus and other organic materials was collected from the snail habitats. It was air-dried then ground into powder. We mixed 500 g powdered soil with 500 ml water to make a thick soil paste about 2 cm thickness, which was placed all over the bottom of each earthen bowl. The snail can produce eggs in the soil paste.

Porcelain enamel trays (35 cm x 45 cm x 1,575 cm) were used to test the efficacy of spraying and powdering. Powdered soil (2,000 g) was used to make a 3–5 cm thick soil paste covering the bottom of each tray. The soil was kept in a 30% wet state by dewatering with 90% ethanol and subsequently bringing to a 30% wet state by adding water. Aquaria (15 cm diameter and 12 cm height containing 2 L test solution was used in the lab to test for toxicity to fishes.

Animals: Adult *O. hupensis* (5.5 to 7.5 mm in length) were collected locally and used as experimental animals. After being rinsed in dechlorinated tap water and kept for 24 h to acclimate in the lab, snails with higher vitality and similar size (presumably of similar age) were chosen for the experiments.

Snail Eggs: 300 experimental snails were kept in earthenware bowls (25 cm diameter and 10 cm height) for 24 h in order to lay eggs. After 24 h, all adult snails were moved from the bowls. These bowls were then kept at room temperature for 25 d. At 25 d, all newly hatched young were removed and unhatched eggs were collected using a 40-mesh sieve (0.45/380 μm).

Zebra fish of 2–3 cm length were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. These fish were acclimated for one week before testing in aquaria.

Experimental Methods

The molluscicidal effects of LDS were assessed by immersion, spraying, and powdering methods in the lab and field. We also used WPN as the molluscicide control and blank control with dechlorinated tap water in the lab and natural water in the field. All lab experiments were done at a room temperature of 25 ± 1°C.

TABLE 2. Results of analysis of variance for two drugs investigated by immersion tests in the lab.

Resources of Variance	v	SS	MS	F	P
Time	2	39,464.21	19,732.11	1,589.60	< 0.0001
Molluscicide	1	3,438.85	3,438.85	264.57	< 0.0001
Concentration	7	133,292.52	19,041.79	1,465.01	< 0.0001
Error of measurement	64	794.45	12.41	-	-

FIGS. 1–6. Application of molluscicide against *Oncomelania hupensis*. FIG. 1: Immersion in the laboratory; FIG. 2: Immersion in the field; FIG. 3: Spraying in the laboratory; FIG. 4: Spraying in the field; FIG. 5: Powdering in the laboratory; FIG. 6: Powdering in the field.

TABLE 3. Changing trend over time for two drugs investigated by immersion tests in the lab.

Resources of Variance	Time	v	SS	MS	F	P
Time	Linearity	1	37,036.37	37,036.33	429.58	< 0.001
Time* Molluscicide	Linearity	1	2,698.76	2,698.76	31.30	< 0.001
Time* Concentration	Linearity	7	12,646.98	1,806.71	20.96	< 0.001
Error of measurement	Linearity	39	3,362.43	86.22	-	-

TABLE 4. LC_{50} of two drugs at different times.

	LDS			WPN		
Exposure Time (h)	LC_{50}	95% Confidence Interval		LC_{50}	95% Confidence Interval	
24	0.4751	0.2989	0.7758	0.2470	0.1914	0.3148
48	0.0691	0.0517	0.0854	0.0903	0.0692	0.1122
72	0.0568	0.0472	0.0657	0.0400	0.0188	0.0547

Snail Immersion: In the lab, 30 active, mature *O. hupensis* were exposed to LDS and WPN at seven concentrations 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mg/L (Fig. 1). Snail mortalities were observed after 24 h, 48 h, and 72 h respectively. In the control group, the same number of snails was kept in dechlorinated tap water under the same conditions. Mortality was determined by crushing the snails (MOH, 2000). LC_{50} values were calculated by probits.

In the field, seven irrigation and drainage ditches (30 m x 2 m) were selected. Nine nylon bags, each with 30 active mature *O. hupensis*, were used. Three nylon bags were immersed in each concentration group of 0.2, 0.4, 0.8 mg/L. This was repeated for LDS and WPN (Fig. 2). Snail mortality in each trial was observed after 24 h, 48 h, and 72 h.

Snail Spraying: In the lab, the concentrations of LDS and WPN were the same as in the immersion method. Molluscicides were sprayed on each tray (1,000 mL/m^2 dechlorinated tap water with dissolved LDS and WPN respectively) (Fig. 3). Two hundred active mature *O. hupensis* were thus exposed for 1 d, 3 d, and 7 d in the trays. After each such exposure, 50 snails were collected randomly to assess mortality.

In the field, 2,100 m^2 of snail habitat on the Yangtze River flood plain was selected and divided into seven sections. Weeds and vegetation were cut down before spraying. In each section, three groups of *O. hupensis* were sprayed with LDS and WPN, each at concentrations of 0.2 g/m^2, 0.4 g/m^2, and 0.8 g/m^2 (Fig. 4). After 1 d, 3 d, and 7 d, 200 snails were collected randomly in each section to identify mortality.

Snail Powdering: In the lab, concentrations of LDS and WPN were as for the immersion method. The chemicals were mixed with sand (1:4) and the mixture was scattered over each tray (Fig. 5). 200 active mature *O. hupensis* were exposed for 1 d, 3 d, and 7 d in earthenware trays. After 1 d, 3 d, and 7 d, 50 snails were collected to determine mortality.

In the field, a similar terrain and vegetation was selected as for the spraying method. LDS and WPN were mixed with fine sand (at the same concentration as above) before scattering the powder (Fig. 6). For each section, the area used was 300 m^2. After 1 d, 3 d, and 7 d, 200 snails were collected randomly in each section to identify mortality.

Snail Egg Immersion: The concentration of LDS and WPN was the same as in with the immersion method. Fifty snail eggs were put in each 50 mL beaker. The beakers were filled with 50 mL of molluscicide solution for each concentration group. The snail eggs were

TABLE 5. Percent mortality for time and concentration of two drugs investigated by immersion tests in the field.

	LDS			WPN		
Active Concentration (mg/L)	24 h	48 h	72 h	24 h	48 h	72 h
0.2	44.00 ± 2.00	94.67 ± 1.15	100.00 ± 0.00	28.00 ± 2.00	68.67 ± 1.15	90.00 ± 0.00
0.4	91.33 ± 3.06	99.33 ± 1.15	100.00 ± 0.00	62.00 ± 2.00	76.00 ± 2.00	95.33 ± 1.15
0.8	95.33 ± 1.15	100.00 ± 0.00	100.00 ± 0.00	75.33 ± 3.06	95.33 ± 1.15	100.00 ± 0.00
Control	4.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	4.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00

TABLE 6. Results of analysis of variance for two drugs investigated by immersion tests in the field.

Resources of Variance	v	SS	MS	F	P
Time	2	7,522.11	3,761.06	1,962.29	< 0.0001
Molluscicide	1	2,244.50	2,244.50	1,496.33	< 0.0001
Concentration	7	89,152.17	29,717.39	19,811.60	< 0.0001
Error of measurement	32	61.33	1.92	-	-

removed from the solutions after exposure for 24 h, 48 h, and 72 h and then cleaned with dechlorinated tap water. After a 48 h hatching period, the usual hatching time for *O. hupensis*, hatching rate and viability were determined.

Fish Toxicity: The half-static state method (NSMC, 1995) was used to assess toxicity. The concentration of LDS and WPN was the same as in the immersion method. Ten fish were exposed in each concentration for 8 h, 24 h, 48 h, 72 h, and 96 h and the mortality at each time was recorded. Fish death was recorded when a fish floated on the surface of the water and did not move even after stimulating the fish tail with a needle. LC_{50} was calculated by probits.

Cost Comparison: The cost comparison of LDS with WPN was done. The unit cost and the total dosage needed to treat the same snail habitats of one hectare were compared for the two molluscicides.

Statistical Methods

Statistical analysis was performed with SAS statistical software, version 8.02. The *P* value accepted for statistical significance was $P < 0.05$. Analysis of variance (ANOVA) was used to analyze repeated measurement data.

RESULTS

Snail Immersion: In the lab, there was significantly greater mortality using LDS at 24 h and 48 h when the concentration of the chemicals was 0.4 mg/L (24 h, LDS vs. WPN, $t = 14.313$, $P < 0.001$; 48 h, LDS vs. WPN, $t = 149$, $P < 0.001$), but there was no significant difference at 72 h (Table 1). At 72 h, all the snails were dead and at concentrations above 0.4 mg/L. There was 100% mortality at each time interval. All three factors (time, molluscicide and concentration) showed significant differences (Table 2). Snail mortality increased with time and concentration, exposure time getting longer and concentration increasing (Table 3). LC_{50} vales for immersion at room temperature (25 ± 1°C), LC_{50} for 48 h exposure, LDS was 0.0691 mg/L. WPN was 0.0903 mg/L (Table 4).

TABLE 7. Percent mortality for time and concentration of two drugs investigated by spraying tests in the lab.

Active Concentration (g/m^2)	LDS 1 d	LDS 3 d	LDS 7 d	WPN 1 d	WPN 3 d	WPN 7 d
0.05	32.67 ± 15.28	48.67 ± 11.55	71.33 ± 12.86	31.33 ± 15.28	44.67 ± 11.55	65.33 ± 16.28
0.1	45.33 ± 11.02	75.33 ± 12.70	87.33 ± 5.77	45.33 ± 12.86	66.67 ± 16.77	78.00 ± 10.00
0.2	51.33 ± 16.17	88.00 ± 7.21	92.00 ± 5.29	51.33 ± 16.29	75.33 ± 11.02	84.00 ± 10.00
0.4	65.33 ± 11.02	95.33 ± 4.16	97.33 ± 2.31	63.33 ± 16.29	83.33 ± 9.24	89.33 ± 5.77
0.8	79.33 ± 7.57	100.00 ± 0.00	100.00 ± 0.00	79.33 ± 11.02	95.33 ± 2.31	97.33 ± 2.30
1.6	86.67 ± 8.08	100.00 ± 0.00	100.00 ± 0.00	94.00 ± 3.46	100.00 ± 0.00	100.00 ± 0.00
3.2	95.33 ± 2.31	100.00 ± 0.00	100.00 ± 0.00	97.33 ± 4.62	100.00 ± 0.00	100.00 ± 0.00
Control	2.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	2.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00

TABLE 8. Results of analysis of variance for two drugs investigated by spraying tests in the lab.

Resources of Variance	v	SS	MS	F	P
Time	2	7,522.11	3,761.06	1,962.29	< 0.0001
Molluscicide	1	2,244.50	2,244.50	1,496.33	< 0.0001
Concentration	3	89,152.17	29,717.39	19,811.60	< 0.0001
Error of measurement	32	61.33	1.92	-	-

In the field, The two-sample t test showed that the difference between LDS and WPN were statistically significant at 24 h and 48 h, (24 h, LDS vs. WPN, t = 14.914, *P* < 0.001; 48 h, LDS vs. WPN, t = 17.500, *P* < 0.001) and displayed no statistical significance in 72 h (72 h, LDS vs. WPN, t = 7.00, *P* = 0.02) (Table 5). All results are statistically significant for factors of time, molluscicide and concentration (Table 6).

Snail Spraying: In the lab, the result of two-sample t tests showed there were no statistical differences between LDS and WPN at 1 d, 3 d, and 7 d (1 d, LDS vs. WPN, t = 0, P = 1.000; 3 d, LDS vs. WPN, t = 3.500, P = 0.073 and 7 d, LDS vs. WPN, t = 2.00, P = 0.184) (Table 7).

Each of the three factors (time, molluscicide and concentration) showed statistically significant variation (Table 8).

In the field, two-sample t test showed that the differences between LDS and WPN were statistically significant at 24 h and 48 h (24 h, LDS vs. WPN, t = 4.541, *P* = 0.01; 48 h, LDS vs. WPN, t = 2.789, *P* = 0.049) but not at 72 h (Table 9). Each of the three factors (time, molluscicide and concentration) showed statistically significant variation (Table 10).

Snail Powdering: In the lab, the results of two-sample t tests showed that the differences between LDS and WPN were not statistically significant at 1 d, 3 d, and 7 d (1d, LDS vs. WPN, t = 0, *P* = 1.000; 3 d, LDS vs. WPN, t = 1.265, *P* = 0.275 and 7 d, LDS vs. WPN, t = 0, *P* = 1.000) (Table 11). Time and concentration showed no statistically singificant variation, whereas mollusicide did show such a variation (Table 12).

In the field, two-sample t tests showed statistically significant differences between LDS and WPN at 1 d (LDS vs. WPN, t = 4.541, *P* = 0.01) and 3 d (LDS VS WPN, t = 2.789, *P* = 0.049) but not at 7 d (LDS vs. WPN, t = 0.929, *P* = 0.406) (Table 13). Each of the three factors (time, molluscicide and concentration) showed statistically significant variation (Table 14). As seen in Table 14, that all of them had statistical significance at the level of time, molluscicide, and concentration.

Snail Egg Immersion: When *O. hupensis* eggs were exposed to LDS and WPN at concentrations of 0.8 mg/L for 24 h, 48 h, and 72 h, two-sample *t* tests showed that the differences between LDS and WPN were statistically significant at 24 h (24 h, LDS vs. WPN,

TABLE 9. Percent mortality for time and concentration of two drugs investigated by spraying tests in the field.

Active Concentration (g/m^2)	LDS			WPN		
	1 d	3 d	7 d	1 d	3 d	7 d
0.2	21.03 ± 1.63	78.77 ± 6.47	90.53 ± 1.53	19.17 ± 1.69	60.20 ± 5.04	92.07 ± 1.53
0.4	55.63 ± 2.95	91.77 ± 5.02	96.53 ± 3.23	52.80 ± 6.08	82.40± 5.00	95.77 ± 1.06
0.8	91.80 ± 4.23	96.63 ± 2.97	99.27 ± 1.27	72.20 ± 6.16	91.30 ± 1.47	97.47 ± 3.11
Control	3.57 ± 0.31	4.43 ± 0.40	4.33 ± 0.23	3.57 ± 0.31	4.43± 0.40	4.33 ± 0.23

TABLE 10. Results of analysis of variance for two drugs investigated by spraying tests in the field.

Resources of Variance	v	SS	MS	F	P
Time	2	13,624.06	6,812.03	799.09	< 0.0001
Molluscicide	1	429.25	429.25	27.00	< 0.0001
Concentration	3	80,511.84	26,837.28	1,688.09	< 0.0001
Error of measurement	32	272.79	8.52	-	-

t = 16.000, $P = 0.004$), but not at 48 h and 72 h (Table 15). Each of the three factors (time, molluscicide and concentration) showed statistically significant variation. As shown, all had statistics significance at the level of time, molluscicide and concentration (Table 16).

Fish Toxicity: At a room temperature of 23–25°C, for fish exposed to 0.4 g/m^2 for 24 h, the mortality produced by LDS and WPN were 40% and 100% respectively. The LC_{50} results for 24 h exposure to LDS and WPN were 0.5657 mg/L and 0.2049 mg/L respectively (Table 17).

Cost Comparison: The costs of the two molluscicides were compared by assuming an immersion treatment of the same area in the field. For 10% LDS, 4 g/m^2 × 10,000 m^2 = 40,000 g (40 kg/hectare), 10 yuan/kg × 40kg = 400 yuan/hectare. For 50% WPN, 2 g/m^2 × 10,000 m^2 = 20,000 g (20 kg/hectare), 35 yuan/kg × 20 kg = 700 yuan/hectare. Thus, the treatment cost for LDS was less than that of WPN (Table 18).

DISCUSSION

While many molluscicides are effective in killing snails, there are problems regarding both toxicity to non-target animals and degradation (Zidan, 2002; Graebing et al., 2004b; Xu et al., 2006). Some molluscicides have been reported as being effective in killing snails and being less toxic to fish, such as the fruit of *Solanum xanthocarpum*, niclosamide- based chemicals (Wei et al., 2002), and polymeric niclosamide formulations against *Biomphalaria alexandrina* snails (Kenawy, 2004). The mixing of nictinalinate with niclosamide not only increase effectiveness in killing snails, but also reduces toxicity to fish (Cai et al., 2002; Yuan et al., 2005b, 2007; Xu et al., 2007; Dai et al., 2008).

Currently, results with LDS show that at concentrations of 0.4 mg/L, and 72 hour exposure by immersion, snail mortality values for LDS and WPN were 100% and 96.70% respectively in the lab, and 100% and 95.33% respectively in the

TABLE 11. Percent mortality for time and concentration of two drugs investigated by powdering tests in the lab.

Active Concentration (g/m^2)	LDS			WPN		
	1 d	3 d	7 d	1 d	3 d	7 d
0.05	18.67 ± 4.16	30.67 ± 5.77	38.00 ± 10.00	16.67 ± 4.16	23.33 ± 2.31	36.00 ± 10.00
0.1	50.67 ± 10.07	61.33 ± 1.15	71.33 ± 5.77	46.67 ± 10.07	58.00 ± 2.00	67.33 ± 2.31
0.2	64.00 ± 10.58	81.33 ± 1.15	88.67 ± 2.31	63.33 ± 11.55	74.00 ± 5.29	84.67 ± 5.77
0.4	80.00 ± 8.72	87.33 ± 2.31	91.33 ± 3.06	74.00 ± 10.39	81.33 ± 5.03	90.00 ± 5.29
0.8	84.67± 11.02	94.67 ± 1.15	98.00 ± 0.00	84.67 ± 5.77	92.00 ± 3.46	98.00 ± 0.00
1.6	90.67 ± 2.31	100.00 ± 0.00	100.00 ± 0.00	88.67 ± 5.77	97.33 ± 1.15	100.00 ± 0.00
3.2	96.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	94.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
Control	1.67 ± 0.58	3.33 ± 0.58	4.00 ± 0.00	1.67 ± 0.58	3.33 ± 0.58	4.00 ± 0.00

TABLE 12. Results of analysis of variance for two drugs investigated by powdering tests in the lab.

Resources of Variance	v	SS	MS	F	P
Time	2	4,421.06	2,210.53	103.38	< 0.0001
Molluscicide	1	205.44	205.44	5.38	0.0268
Concentration	7	15,4133.97	22,019.14	577.13	< 0.0001
Error of measurement	64	1,368.44	21.38	-	-

TABLE 13. Percent mortality for time and concentration of two drugs investigated by powdering tests in the field.

	LDS			WPN		
Active Concentration (mg/L)	1 d	3 d	7 d	1 d	3 d	7 d
0.2	47.40 ± 6.46	74.13 ± 4.63	84.93 ± 7.18	34.30 ± 4.36	58.87 ± 5.35	85.33 ± 3.52
0.4	65.00 ± 6.37	93.50 ± 3.77	99.33 ± 1.15	60.43 ± 5.61	82.07± 4.14	94.60 ± 1.42
0.8	83.07 ± 12.08	98.93 ± 1.01	100.00 ± 0.00	72.73 ± 6.03	91.53 ± 1.00	100.00 ± 0.00
Control	2.93 ± 0.25	3.97 ± 0.76	3.77 ± 0.45	3.33 ± 0.51	3.97± 0.76	4.07 ± 0.23

TABLE 14. Results of analysis of variance for two drugs investigated by powdering tests in the field.

Resources of Variance	v	SS	MS	F	P
Time	2	13,624.06	6,812.03	799.09	< 0.0001
Molluscicide	1	429.25	429.25	27.00	< 0.0001
Concentration	3	80,511.84	26,837.28	1,688.09	< 0.0001
Error of measurement	32	272.79	8.52	-	-

TABLE 15. Percent mortality of snail eggs for time and concentration of two drugs investigated by immersion tests in the lab.

	LDS			WPN		
Active Concentration (mg/L)	24 h	48 h	72 h	24 h	48 h	72 h
0.05	9.00 ± 5.29	14.33 ± 5.13	29.67 ± 5.03	8.67 ± 5.51	13.33 ± 12.74	25.67 ± 3.06
0.1	14.00 ± 5.29	34.67 ± 0.58	52.67 ± 2.52	13.67 ± 5.51	28.33 ± 15.28	49.00 ± 5.29
0.2	33.00 ± 1.73	57.33 ± 2.08	80.67 ± 1.15	24.33 ± 5.13	52.67 ± 2.31	75.00 ± 4.00
0.4	63.67 ± 4.04	92.33 ± 2.08	94.00 ± 3.46	55.00 ± 0.00	82.33 ± 2.08	89.00 ± 1.73
0.8	75.00± 0.00	93.00 ± 2.65	96.33 ± 1.15	64.33 ± 1.15	90.67 ± 1.15	95.00 ± 0.00
1.6	85.00 ± 0.00	95.67 ± 0.58	100.00 ± 0.00	81.00 ± 1.73	95.00 ± 0.00	100.00 ± 0.00
3.2	90.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	85.67 ± 1.15	100.00 ± 0.00	100.00 ± 0.00
Control	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00

TABLE 16. Results of analysis of variance for two drugs investigated by immersion tests in the lab.

Resources of Variance	v	SS	MS	F	P
Time	2	14,250.167	7,125.08	875.81	< 0.0001
Molluscicide	1	416.84	416.84	14.51	< 0.0001
Concentration	7	17,1157.44	24,451.06	851.09	< 0.0001
Error of measurement	64	520.67	8.14	-	-

field. We showed that the effect of using these two molluscicides is the same, but LDS had a significantly lower toxicity to fish. Moreover the cost of LDS is much lower than that of WPN. The cost of WPN is now 35 Chinese Yuan per kilogram while LDS is 10 Chinese Yuan per kilogram. In addition, as the production process for LDS is simpler than for WPN, production costs could be reduced by a factor approximately ten. Hence, production costs and application fees are both decreased. For example, the cost of 50% WPN is 35 Yuan/per kilogram and 10% LDS is 10 Yuan/per kilogram. As killing the snails by immersion method over the same area of one hectare, using 50% WPN with 2 mg/L needs 20 kg and 700 Chinese Yuan, while using 10% LDS with 4 mg/L needs 40 kg and 400 Chinese Yuan. It is clear that the cost of LDS is 42% lower than the cost of WPN.

TABLE 17. Fish toxicity LC$_{50}$ for LDS and WPN in the lab.

Name of Drug	Time (h)	LC$_{50}$ (mg/L)	95% Confidence Interval	
			Lower Limit	Upper Limit
LDS	8	1.7493	1.1411	3.5128
	24	0.5657	0.4091	0.7823
	48	0.4666	0.3391	0.6301
	72	0.4666	0.3391	0.6301
WPN	8	0.4820	0.3081	0.7448
	24	0.2049	-	-
	48	0.1381	0.0964	0.1968
	72	0.0851	0.06	0.1179

The molluscicidal efficacy of LDS was observed in different concentrations and different exposure times in the lab. The results show that snail mortality increased with both time and concentration. The effectiveness of LDS was clearly related to time of exposure.

The fish toxicity tests showed that for fish exposed to 0.4 g/m^2 for 24 h, the LC$_{50}$ of LDS was 1.7 times that of WPN (LDS 0.5657 mg/L; WPN 0.2049 mg/L). This means LDS is safer for the fish than WPN.

In our opinion, LDS is a reliable molluscicide that can be readily used in different habitats and at low cost. We recommend it as the molluscicide of choice for use in a variety of habitats.

TABLE 18. Comparing the cost between LDS and WPN.

Content	10% LDS	50% WPN	Gradient with LDS to WPN (%)
Original materials	abio-alkali	ethanol amine	
Price (RMB per yuan/per ton)	3,000	17,000	Decline 82.35
Commercial price (RMB yuan/per ton)	56,000	70,000	Decline 20.00
Packing materials	abio-salt	diatomite	
Price of packing (RMB yuan/per ton)	500	350	Augment 42.85
Price of commercial molluscicide (RMB yuan/per ton)	10,000	35,000	Decline 71.42
Commercial molluscicide			
Using dosage (kg/hectare)	40/1	70/1	
Market price (RMB yuan/hectare)	400/1	700/1	Decline 42.85

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